Immunohistochemical assessment of human herpesvirus 8 infection in primary central nervous system large B cell lymphomas

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Abstract

***Background/Aims*—Primary central nervous system large B cell lymphomas (PCNSLs) are frequently associated with Epstein-Barr virus (EBV) in patients with AIDS and less frequently in those without AIDS. Human herpesvirus 8 (HHV-8) has been detected in these tumours by the polymerase chain reaction (PCR) at low copy number, suggesting its presence in a cell compartment other than the malignant one. The aim of this study was to use immunohistochemistry to assess HHV-8 infection in a series of PCNSLs from patients with and without AIDS.**

***Methods*—The antibody LN53, which reacts with the latent nuclear antigen 1 (LNA1) of HHV-8, was used on tissue sections from 35 patients (17 with and 18 without AIDS) with PCNSL. In addition, DNA was available for PCR (open reading frame 26 (ORF 26), ORF 72, ORF 75) in three patients (two without AIDS, one with AIDS).**

***Results*—None of the 35 cases contained either DNA sequences or LNA1 positive cells.**

***Conclusions*—These results confirm the lack of HHV-8 infection in tumour cells of PCNSL. In addition, HHV-8 infected bystander cells do not express LNA1 latent protein in this setting.**

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Keywords: human herpesvirus 8; lymphoma; AIDS; human immunodeficiency virus

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Primary central nervous system large B cell lymphomas (PCNSLs) of patients with AIDS are associated with Epstein-Barr virus (EBV) in almost all cases. The incidence of EBV infection is much less frequent in PCNSL of patients without AIDS.[1] Human herpesvirus 8 (HHV-8) is often found in multicentric Castleman's disease and primary effusion lymphoma seen during AIDS.[2 3] There have been indications that it may be implicated in PCNSL, although this has not yet been confirmed.[4–8] Several studies relying on the polymerase chain reaction (PCR) detection of viral nucleic acids have described the presence of the viral genome in tissues involved by PCNSL, but at a very low copy number,[6 7] suggesting that HHV-8 infects bystander lymphoid cells but not lymphoma cells.

In this study, we sought to determine whether HHV-8 was present in PCNSLs from 17 patients with and 18 patients without AIDS. In all cases, we used immunohistochemistry with the LN53 antibody, which is directed against the latent nuclear antigen 1 (LNA1) of HHV-8, to characterise the cell compartment infected by the virus. In three cases, a very sensitive PCR technique was also used.

Materials and methods

Thirty five paraffin wax embedded formalin or Bouin fixed postmortem (n = 11) or biopsy (n = 24) specimens of brain lymphomas from patients with (n = 17) and without AIDS (n = 18) were collected between 1993 and 2000 from the files of the pathology departments involved in our study (Rangueil and Purpan Hospitals (Toulouse) and Pellegrin Hospital (Bordeaux)). These patients were selected retrospectively because sufficient pathological material was available to be considered representative of the tumour. The mean age at presentation was 72 years (range, 55 to 84) in patients without AIDS, whereas the mean age at presentation was 36 years (range, 32 to 39) in patients with human immunodeficiency virus (HIV) infection. All lymphomas were of the B cell phenotype and classified as diffuse large B cell lymphomas according to the REAL classification system.[9] The diagnosis was established according to immunomorphological criteria.

HHV-8 was investigated immunohistochemically with the use of a rat monoclonal anti-LNA1 antibody (LN53; ABI, Colombia, Maryland, USA), according to a previously published protocol.[10] Specimens of Kaposi's sarcoma were used as positive controls.

The absence of HHV-8 was confirmed by PCR in a case of HIV positive PCNSL and in two patients without AIDS. The other cases were not studied because the tissues were fixed in a picric acid based fixative and the DNA was not intact. The details of the PCR assay have been published previously.[11] We chose the primer sets described within the open reading frame 26 (ORF 26), ORF 75, and ORF 72.[11]

Results

The purpose of our study was to determine by means of immunohistochemistry and molecular biology techniques the presence and the localisation of HHV-8 in PCNSL.

We could not detect HHV-8 LNA1 protein by immunohistochemistry in the lymphoma cells of the 35 cases. Similarly, HHV-8 DNA sequences were not detected in the two patients without AIDS or the HIV positive case.

Discussion

Several studies have looked for HHV-8 infection in PCNSL using different PCR techniques. Variable and controversial results were found according to the technique used. Studies have mainly concerned patients with AIDS. In 33 patients with AIDS and PCNSL, Antinori *et al* found no sign of HHV-8 infection in cerebral tissue by means of single step PCR and nested PCR, with the exception of one patient who was also affected by Kaposi's sarcoma.[4] Morgello *et al*, who analysed 27 EBV positive AIDS related PCNSL samples for HHV-8 sequences found only one tumour with detectable HHV-8 sequences. This tumour arose in a patient with a history of Kaposi's sarcoma.[8] In 13 patients (10 with AIDS and three without), Mikol *et al* detected HHV-8 DNA in four of 10 AIDS and two of three non-AIDS tumours.[1] Lastly, using a sensitive nested PCR technique to study 36 postmortem and biopsy specimens of brain lymphoma, Corboy *et al* found that 56% of PCNSLs contained HHV-8 DNA.[7] The percentage of PCNSL specimens with HHV-8 DNA was similar in HIV positive and HIV negative patients. These data are consistent with another report from Gaidano *et al*, who investigated HHV-8 infection in 31 individuals with PCNSL (16 with AIDS, 15 without).[6] All subjects scored negative by single step PCR but, using nested PCR, only 16 were devoid of HHV-8 sequences. This last study underlines the fact that all these data must be interpreted with caution. Indeed, our negative results and the negativity of HHV-8 DNA detection by single step PCR suggest that small amounts of virus are present in patients with PCNSL. In primary effusion lymphomas, HHV-8 is mainly latent and thus expresses the LNA1 protein.[10] The lack of this protein in the lymphoma cells of PCNSL strongly suggests that the virus does not play a role in the pathogenesis of these tumours, irrespective of the HIV status of the patients.

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